ISOLATION, CHARACTERIZATION AND BIOLOGICAL APPLICATIONS OF BIOACTIVE COMPOUNDS FROM ENDOPHYTIC MICROBES

A SYNOPSIS

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1. INTRODUCTION

In the last few decades, the research on biologically active compounds have brought an attention in the field of healthcare and pharmaceutical industries due to their biological importance. Bioactive compounds are termed as the nutraceutical/therapeutic compounds that holds versatile applications for the human welfare [1].

Endophytic microorganisms are complex eukaryotes in association with plants. They are usually living in parts of plants like roots, stem, leaves. Plant-associated microorganisms are symbiotically association with plants and known as scientifically termed as endophytic microbes [2]. These endophytic microbes are present both internally and externally in the plant and produce plant-like bioactive compounds with therapeutic values. Those plant-associated microbes utilize the secondary metabolites from the plants and defense plants against various stress conditions, and pathogens (predators) [3]. These endophytic microbes are majorly noted and given more importance for the production of several novel bioactive compounds. Thus, the endophytic microbes which are the producers of the various biologically active compounds are pharmaceutically more important. The endophytic microbes from *Phyllanthus niruri, Cynodon dactylon, Tribulus terrestris* were some plants with medicinal values. These plants also have endophytic microbes within them that acts as source of producing various biologically active compounds [4].

P. niruri L. is a well-established medicinal herb that has been utilized both traditionally and scientifically [5]. The existence of numerous strong bioactive chemicals in *P. niruri* L. was shown by phytochemical screening which can be exploited to make effective herbal medications. *P. niruri* L. crude extract has a number of pharmacologically important phytocompounds. It has long been recognized as safe and efficient treatment for liver problems [6].

Cynodon dactylon, often known as “Durva Grass”, “Bermuda grass”, “Dog’s Tooth grass”, “Bahama grass”, “Devil’s grass”, “Couch grass”, “Indian Doab”, “Scutch grass”, “Dhub”, and “Arugampul”, is a plant of immense religious significance that is reportedly used in the Ayurvedic, Siddha, Unani, Nepalese, and Chinese medical systems. *C. dactylon* extract has been successfully tested in preclinical studies for its antitumor activity [7].

Tribulus terrestris L. (caltrop family: Zygophyllaceae) is a plant that has been used for a variety of potential health benefits for many years. It is widely distributed

around the world. *T. terrestris* L. has been used for centuries in Ayurveda to treat impotence, venereal diseases, and sexual debility [8].

The bioactive compounds act as good source for treating cancer cells. Notably, bioactive compounds from endophytic organism are currently been used as therapies in the worldwide. The main scope of these bioactive compounds is to replace current synthetic drugs. Because, these synthetic drugs showed toxic side effects, and non-selected distribution of other healthy cells and induce oxidative-stress in cells. Thus, bioactive compounds are mainly involved in the reduction of the reactive oxygen species (ROS), which is mainly responsible for the treating the cancer [9].

2. REVIEW LITERATURE

2.1 Bioactive compounds

Bioactive compounds are naturally occurring compounds that holds various medicinal properties. The major properties of these compounds are anti-inflammatory, anti-carcinogenic, anti-oxidant, anti-coagulant, anti-diabetic, anti-microbial, and cardiovascular, neuro protective properties. Bioactive compounds are produced by bacteria, fungi, algae, plants as secondary metabolites for their own protection against predators, Jalgaonwala and Mahajan [10]. These bioactive compounds obtain through various extraction methods and purified by chromatographic techniques. The majority of the currently used medicines are synthetic drugs. These drugs are costly and exhibited severe adverse effects, non-selective distribution, early elimination on normal tissues, while using bioactive compounds from natural sources are renewable with low cost, less side effects, Khalil *et al.* [11].

2.2 Bioactive compounds from endophytic microbes

Bacteria and fungi are complex microorganisms present in soil, plants, sometimes in water. Some of microbes are found symbiotically associated with plants and known as endophytic microorganisms. Endophytic microbes are present in plants and produce plant based metabolic compounds with therapeutic values. Those microbes utilize the secondary metabolites from the plants and defense plants against various stress conditions, pathogens. Endophytic microbes are noted and given more importance for the production of novel bioactive compounds, Yu *et al*. [12].

2.3 Extraction and characterization of bioactive compounds

The presence of these biologically active compounds is very low and produced inside the microbial cell structure. Thus, it requires more technical extraction methods for complete extraction/separation of compounds from endophytic microbes. Most of the laboratories followed conventional extraction methods for extraction of compounds, Selvaraj *et al*. [13].

The main scope of these bioactive compounds is to replace synthetic drugs. Cinnamic acid, Plinabulin, Naringin, Rutin, Kaempferol, Furfural, Quercetin are some of bioactive compounds derived from natural sources with diversified structures. The, structural characterization was helpful in finding novel compounds. The structural characterization of bioactive compounds can be performed using Mass spectrometry, x-ray photoelectron spectroscopy, scanning electron microscopy, NMR. Bioactive compounds are the leading sources of medicines for curing various disorders. They are totally natural derived compounds that can bind to target, act as future medicine with not any adverse effects, Selvaraj *et al*. [14].

2.4 Bioactive compounds for cancer treatment

Interestingly, endophytic microbial bioactive have received a lot of interest recently because of their ability to produce novel materials and goods with improved therapeutic potential. Therefore, bioactive compounds from endophytic microbes serves as natural chemotherapeutic agents for cancer and in promoting human health, Kharwar *et al*. [15].

3. OBJECTIVES

- To isolate the endophytic microbes from the plants *Phyllanthus niruri*, *Cynodon dactylon*, *Tribulus terrestris*
- To extract the bioactive compounds from the endophytic microbes
- Molecular identification of the endophytic microbes based on their bioactive compounds profile
- Characterization studies of the extract of the isolates (GC-MS)
- *In silico* Molecular Docking Studies of bioactive compounds from the endophytic microbes

- To analyze the biological properties of the bioactive compounds– anti-oxidant and anti-cancer studies

4. RESEARCH GAP IDENTIFICATION

With increase in various cancer types, it was essential to find natural medications with lower side effects to treat cancer. Numerous research works done in this field to find the bioactive compounds and their biological activities, but none proved their mechanism of action to kill the cancer cells. In addition to that, first time we try to use endophytic exopolysaccharides for drug delivery application.

5. ORGANIZATION OF THE THESIS

Chapter 1

Chapter 1 explains about the introductory part of the bioactive compounds. We have discussed about the natural bioactive compounds from the medicinal plants. The endophytic bacteria, fungi, algae produce bioactive compounds as a part of their secondary metabolites. They are obtained by isolation, extraction methods and purified by precipitation techniques. The notable property of the bioactive compounds was had been existing as the possible source of anticancer medications. These endophytic microbial bioactive compounds also exhibit antibacterial, antiviral, anti-inflammatory, and immunosuppressive properties

Chapter 2

Chapter 2 illustrates the review of literature related to the endophytic microbes and their compounds for cancer treatment. Several studies indicates the therapeutic value of bioactive compounds for anti-oxidant and anti-cancer properties. Thus, the endophytic microbes which are in production of various biologically active compounds are pharmaceutically more important. These compounds are observed to contain enormous medicinal values naturally.

Chapter 3

Chapter 3 analyse the anti-oxidant and anti-cancer properties of the bioactive compounds from *Phyllanthus niruri* L. Endophytic fungi from root of *Phyllanthus niruri* L. was isolated. The fungal compounds were extracted. The phytochemical profiling of endophytic fungal extract was investigated and characterized by GC-MS analysis whereby thirteen compounds were detected. Among them, 2-methyl-7-phenylindole showed good binding affinity and better interaction with higher docking score -8.8 kcal mol^{-1} in 3POZ (EGFR). The antioxidant potential

of the ethyl acetate fraction of the endophytic fungal extract was the greatest. Furthermore, in HepG2 cells, endophytic fungal extract caused apoptosis via the formation of elevated amounts of ROS, mitochondrial transmembrane potential damage, and nucleus morphological abnormalities (Figure 1). The novel endophytic fungi *C. geniculata* from *P. niruri* L. may become a potential anti-oxidant and anticancer agent.

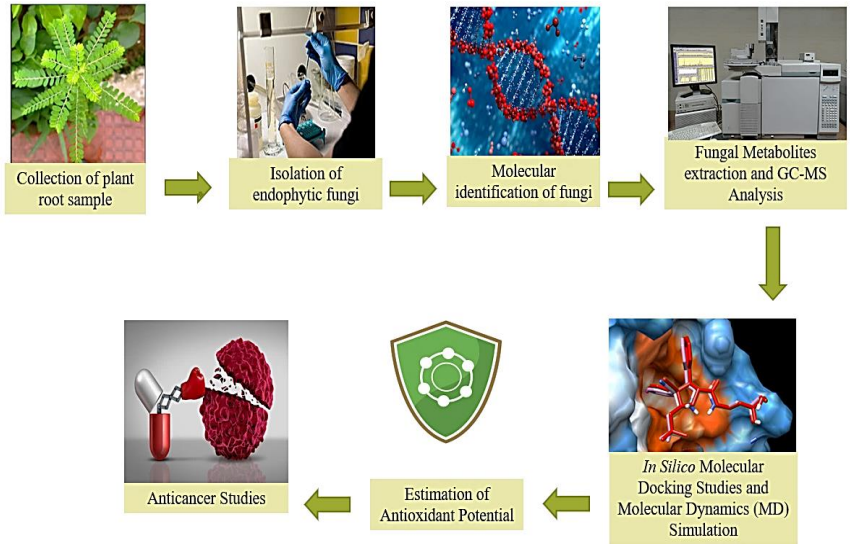
Collection of plant root sample
Isolation of endophytic fungi
Molecular identification of fungi
Fungal Metabolites extraction and GC-MS Analysis
In Silico Molecular Docking Studies and Molecular Dynamics (MD) Simulation
Estimation of Antioxidant Potential
Anticancer Studies

Figure 1: Anti-cancer activity of bioactive compounds from *C. geniculata*

Chapter 4

Chapter 4 analyse the anti-oxidant and anti-cancer properties of the bioactive compounds from *Cynodon dactylon* L. *A. flavus* is an endophytic fungus isolated from the root of *C. dactylon*, and its ethyl acetate extract demonstrates significant antioxidant and anticancer potential against breast cancer (MCF-7) cells. The profiling of bioactive compounds from ethyl acetate extract of endophytic fungus was detected through GC-MS analysis, and 9 bioactive compounds were identified. Among them, 3 bioactive compounds, namely, 2,4,7-Trinitrofluorenone, and 3,6-Bis (N-formamido) carbazole exhibited the highest binding affinities, -9.50, and -9.00 Kcal mol^{-1}, respectively, against the breast cancer receptor HER2.

Subsequently, molecular dynamics simulation studies confirmed their stability and the intermolecular interactions of ligands-receptor complexes. Additionally, the cytotoxic effects of *A. flavus* extract showed *a* significant reduction of cellular viability of MCF-7 cells with an IC50 of 16.25 µg mL^{-1}. The data also highlight that the ethyl acetate fraction of *A. flavus* causes apoptosis in MCF-7 cells through the generation of

higher ROS, nuclear material damage, and dissipation of mitochondrial transmembrane potential (Figure 2).

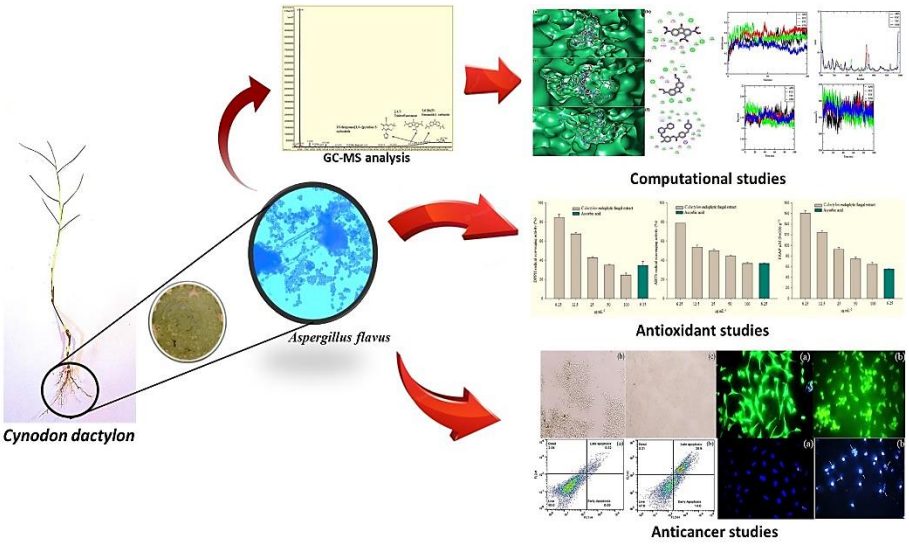

Figure 2: Anti-cancer activity of bioactive compounds from *A. flavus*

Chapter 5

Chapter 5 analyse the anti-oxidant and anti-cancer properties of quercetin loaded exopolysaccharide nanoparticles from *Tribulus terrestris* L. The isolated endophytic bacterial EPS was successfully used for drug delivery applications. Initially, the EPS-producing endophytic bacteria was screened from the roots of *Tribulus terrestris* L. and it was confirmed as *Pseudomonas otitidis*. The producing EPS from the bacterial culture was isolated through the ice-cold ethanol precipitation method, and partially characterized and conformed via FTIR, NMR and their reducing sugar and protein contents were also estimated. In this regard, quercetin-loaded EPS NPs was fabricated through the precipitation method. The fabricated quercetin-loaded NPs characterized by FTIR, XRD, DLS, and SEM for their functional integrity, physical properties, zeta potential, size distribution and morphological characteristics and confirmed.

Further, the drug release studies of the fabricated quercetin-loaded EPS NPs were performed via varied physiological buffer medium (acetate buffer (pH 3.5 and 5.0) and PBS (pH 7.2)). The 82.523±0.854% quercetin was released from quercetin-loaded EPS NPs at 25 h in an acetic medium (pH 5.0). The result of drug release data was that quercetin-loaded EPS NPs fit the first-order kinetic model. In addition, the quercetin-loaded EPS NPs showed significant antioxidant and anticancer properties.

The IC_{50} value of quercetin-loaded EPS NPs found to be 14.313 μg mL^{-1} against MCF-7 cells. The studies further demonstrated that quercetin-loaded EPS NPs triggered apoptosis in MCF-7 cells by excessive production of ROS, damaging nuclear material, and depleting mitochondrial transmembrane potential (Figure 3). Thus, quercetin-loaded EPS NPs serves as the significant antioxidant and anticancer agent through *in vitro* methods. However, structural investigation of the ESP required for further validation and applied for other biological applications.

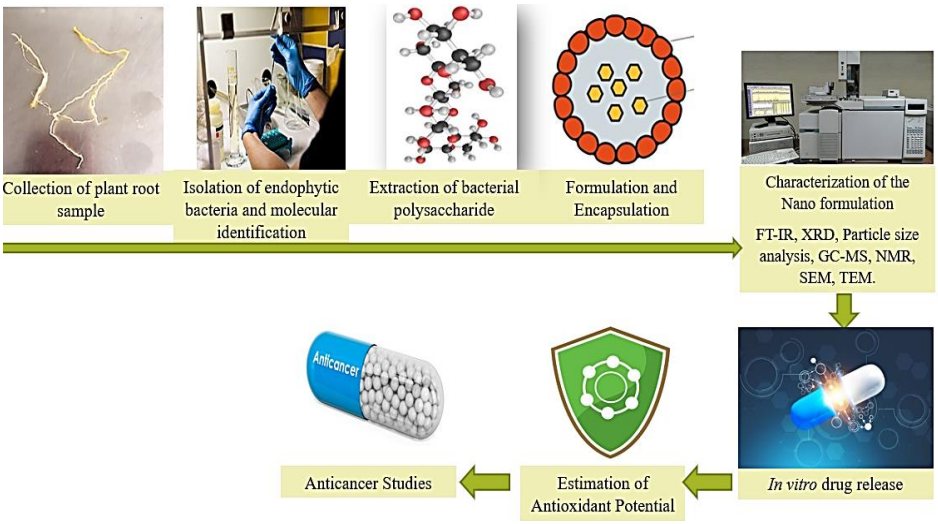
Collection of plant root sample
Isolation of endophytic bacteria and molecular identification
Extraction of bacterial polysaccharide
Formulation and Encapsulation
Characterization of the Nano formulation
FT-IR, XRD, Particle size analysis, GC-MS, NMR, SEM, TEM.
In vitro drug release
Estimation of Antioxidant Potential
Anticancer Studies
Anticancer

Figure 3: Anti-oxidant and anti-cancer properties of quercetin loaded exopolysaccharide nanoparticles from *Pseudomonas otitidis*

Chapter 6

The chapter 6 explore the conclusion of the present work which highlights the critical aspects of the bioactive compounds from medicinal plants for treating cancer. Thus, bioactive compounds from plants *Phyllanthus niruri*, *Cynodon dactylon*, *Tribulus terrestris* were extracted from their endophytic microbes. The bioactive compounds from *Phyllanthus niruri*, *Cynodon dactylon*, and polysaccharide (Quercetin encapsulated) from *Tribulus terrestris* were observed to contain anti-oxidant properties (DPPH, ABTS, FRAP). The Anti-cancer activity was analyzed through MTT, Ao/EtBr staining, MMP, DAPI, ROS assays. From these observations it was proved that the bioactive compounds from the endophytic microbes acts as good source to treat inflammations, to control the over production of ROS and to treat the cancer cells. They also act a drug carrier for drug delivery applications.

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PUBLICATIONS

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